

FILE 'HOME' ENTERED AT 10:53:16 ON 05 SEP 2002

=> FIL BIOSIS MEDLINE CAPLUS EMBASE SCISEARCH  
COST IN U.S. DOLLARS

SINCE FILE ENTRY	TOTAL SESSION
0.21	0.21

FULL ESTIMATED COST

FILE 'BIOSIS' ENTERED AT 10:53:47 ON 05 SEP 2002  
COPYRIGHT (C) 2002 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'MEDLINE' ENTERED AT 10:53:47 ON 05 SEP 2002

FILE 'CAPLUS' ENTERED AT 10:53:47 ON 05 SEP 2002  
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.  
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.  
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'EMBASE' ENTERED AT 10:53:47 ON 05 SEP 2002  
COPYRIGHT (C) 2002 Elsevier Science B.V. All rights reserved.

FILE 'SCISEARCH' ENTERED AT 10:53:47 ON 05 SEP 2002  
COPYRIGHT (C) 2002 Institute for Scientific Information (ISI) (R)

=> s tpa or t-pa of (plasminogen (w) activator)  
MISSING OPERATOR 'OF' (PLASMINOGE'  
The search profile that was entered contains terms or  
nested terms that are not separated by a logical operator.

=> s tpa or t-pa or (plasminogen (w) activator)  
L1 166707 TPA OR T-PA OR (PLASMINOGEN (W) ACTIVATOR)

=> s l1 and (k429? or h417? or d477?)  
L2 6 L1 AND (K429? OR H417? OR D477?)

=> dup rem l2  
PROCESSING COMPLETED FOR L2  
L3 3 DUP REM L2 (3 DUPLICATES REMOVED)

=> d py l3 1-3

L3 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2002 ACS  
PY 1999  
1999

L3 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2002 ACS  
PY 1998  
1998  
1998  
2001  
1999  
2000  
2001

L3 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1  
PY 1990

=> d 3 l3 ab so ti py au

L3 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1  
AB In contrast to most other serine proteases, tissue-type  
\*\*\*plasminogen\*\*\* \*\*\*activator\*\*\* ( \*\*\*t\*\*\* - \*\*\*PA\*\*\* )  
possesses enzymatic activity as the one-chain zymogen form. The hypothesis  
that lysine residues 277 or 416 may be involved in stabilization of an  
active conformation of one-chain \*\*\*t\*\*\* - \*\*\*PA\*\*\* via salt-bridge  
formation with aspartic acid residue 477 was tested by site-directed  
mutagenesis. Four recombinant \*\*\*t\*\*\* - \*\*\*PA\*\*\* mutants were  
constructed. The amidolytic activities of these analogues were compared to  
that of authentic \*\*\*t\*\*\* - \*\*\*PA\*\*\*. Substitution of arginine-275  
provided an analogue ([R275G] \*\*\*t\*\*\* - \*\*\*PA\*\*\* ) resistant to  
plasmin cleavage. The amidolytic activity of [R275G] \*\*\*t\*\*\* - \*\*\*PA\*\*\*

was comparable to that of authentic one-chain [R275L,K277L], in which  
 and so was the activity [R275L,K277L], in which  
 additional substitution of lysine residue 277 was carried out. This  
 suggested that its presence was nonessential for obtaining one-chain  
 activity. In contrast, substitution of lysine  
 residue 416 to obtain [K416S] and [K416S,  
 H417T] resulted in substantial quenching  
 of amidolytic one-chain activity. As expected, the amidolytic activities  
 of the two-chain forms were less affected by the substitution. Involvement  
 of lysine residue 416 in one-chain activity was  
 also indicated by decreased activities of [K416S] and [K416S,  
 H417T] with plasminogen as  
 the substrate. The one-chain activity of the lysine residue 416  
 substitution analogues was partially restored in the presence of fibrin.  
 This could indicate that strong ligands such as fibrin might provide an  
 alternative stabilization of the active conformation of one-chain

SO BIOCHEMISTRY, (1990) 29 (14), 3451-3457.  
 CODEN: BICHAW. ISSN: 0006-2960.  
 TI QUENCHING OF THE AMIDOLYTIC ACTIVITY OF ONE-CHAIN TISSUE-TYPE  
 \*\*\*PLASMINOGEN\*\*\* \*\*\*ACTIVATOR\*\*\* BY MUTATION OF LYSINE-416.  
 PY 1990  
 AU PETERSEN L C; BOEL E; JOHANNESSEN M; FOSTER D

=> d hist

(FILE 'HOME' ENTERED AT 10:53:16 ON 05 SEP 2002)

FILE 'BIOSIS, MEDLINE, CAPLUS, EMBASE, SCISEARCH' ENTERED AT 10:53:47 ON  
 05 SEP 2002

L1 166707 S TPA OR T-PA OR (PLASMINOGEN (w) ACTIVATOR)  
 L2 6 S L1 AND (K429? OR H417? OR D477?)  
 L3 3 DUP REM L2 (3 DUPLICATES REMOVED)

=> s l1 and zymogen

L4 997 L1 AND ZYMOGEN

=> dup rem l4

PROCESSING COMPLETED FOR L4  
 L5 364 DUP REM L4 (633 DUPLICATES REMOVED)

=> s l5 and fibrin

L6 97 L5 AND FIBRIN

=> s l6 and py<=1996

1 FILES SEARCHED...  
 4 FILES SEARCHED...  
 L7 62 L6 AND PY<=1996

=> s l7 and (275 or 276)

L8 2 L7 AND (275 OR 276)

=> d ab 18

L8 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AB In contrast to most other serine proteases, tissue-type  
 \*\*\*plasminogen\*\*\* \*\*\*activator\*\*\* ( \*\*\*t\*\*\* - \*\*\*PA\*\*\* )  
 possesses enzymatic activity as the one-chain \*\*\*zymogen\*\*\* form. The  
 hypothesis that lysine residues 277 or 416 may be involved in  
 stabilization of an active conformation of one-chain \*\*\*t\*\*\* -  
 \*\*\*PA\*\*\* via salt-bridge formation with aspartic acid residue 477 was  
 tested by site-directed mutagenesis. Four recombinant \*\*\*t\*\*\* -  
 \*\*\*PA\*\*\* mutants were constructed. The amidolytic activities of these  
 analogues were compared to that of authentic \*\*\*t\*\*\* - \*\*\*PA\*\*\* .  
 Substitution of arginine- \*\*\*275\*\*\* provided an analogue ([R275G]  
 \*\*\*t\*\*\* - \*\*\*PA\*\*\* ) resistant to plasmin cleavage. The amidolytic  
 activity of [R275G] \*\*\*t\*\*\* - \*\*\*PA\*\*\* was comparable to that of  
 authentic one-chain \*\*\*t\*\*\* - \*\*\*PA\*\*\* , and so was the activity of  
 [R275L,K277L] \*\*\*t\*\*\* - \*\*\*PA\*\*\* , in which additional substitution of  
 lysine residue 277 was carried out. This suggested that its presence was  
 nonessential for obtaining one-chain \*\*\*t\*\*\* - \*\*\*PA\*\*\* activity. In  
 contrast, substitution of lysine residue 416 to obtain [K416S] \*\*\*t\*\*\* -  
 \*\*\*PA\*\*\* and [K416S,H417T] \*\*\*t\*\*\* - \*\*\*PA\*\*\* resulted in  
 substantial quenching of amidolytic one-chain activity. As expected, the  
 amidolytic activities of the two-chain forms were less affected by the

substitution. Involvement of lysine residue 416 in one-chain \*\*\*\*t\*\*\*\* -  
\*\*\*\*PA\*\*\*\* activity was also indicated by decreased activities of [K416S]  
\*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\* and [K416S,H417T] \*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\* with  
 plasminogen as the substrate. The one-chain activity of the lysine residue  
 416 substitution analogues was partially restored in the presence of  
\*\*\*\*fibrin\*\*\*\* . This could indicate that strong ligands such as  
\*\*\*\*fibrin\*\*\*\* might provide an alternative stabilization of the active  
 conformation of one-chain \*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\* .

=> d ab 18 2

L8 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 AB Activation of the \*\*\*\*zymogen\*\*\*\* form of a serine protease is  
 associated with a conformational change that follows proteolysis at a  
 specific site. Tissue-type \*\*\*\*plasminogen\*\*\*\* \*\*\*\*activator\*\*\*\* (  
\*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\*) is homologous to mammalian serine proteases and  
 contains an apparent activation cleavage site at arginine- \*\*\*\*275\*\*\*\* .  
 To clarify the functional consequences of cleavage at arginine- \*\*\*\*275\*\*\*\*  
 of \*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\* , site-specific mutagenesis was performed to  
 convert arginine- \*\*\*\*275\*\*\*\* to a glutamic acid. The mutant enzyme  
 (designated Arg- \*\*\*\*275\*\*\*\* .fwdarw. Glu \*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\*) could  
 be converted to the two-chain form by Staphylococcus aureus V8 protease  
 but not by plasmin. The one-chain form was 8 times less active against the  
 tripeptide substrate H-D-isoleucyl-L-prolyl-L-arginine-p-nitroanilide  
 (S-2288), and the ability of the enzyme to activate plasminogen in the  
 absence of fibrinogen was reduced 20-50 times compared to the two-chain  
 form. In contrast, one-chain Arg- \*\*\*\*275\*\*\*\* .fwdarw. Glu \*\*\*\*t\*\*\*\* -  
\*\*\*\*PA\*\*\*\* has equal activity to the two-chain form when assayed in the  
 presence of physiological levels of fibrinogen and plasminogen.  
\*\*\*\*Fibrin\*\*\*\* bound significantly more of the one-chain form of \*\*\*\*t\*\*\*\*  
- \*\*\*\*PA\*\*\*\* than the two-chain form for both the wild-type and mutated  
 enzymes. One- and two-chain forms of the wild-type and mutated  
\*\*\*\*plasminogen\*\*\*\* \*\*\*\*activators\*\*\*\* slowly formed complexes with  
 plasma protease inhibitors, although the one-chain forms showed decreased  
 complex formation with .alpha.2-macroglobulin. The one-chain form of  
\*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\* therefore is fully functional under physiologic  
 conditions and has an increased \*\*\*\*fibrin\*\*\*\* binding compared to the  
 two-chain form.

=> d ab 18 2 py so ti au

L8 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 AB Activation of the \*\*\*\*zymogen\*\*\*\* form of a serine protease is  
 associated with a conformational change that follows proteolysis at a  
 specific site. Tissue-type \*\*\*\*plasminogen\*\*\*\* \*\*\*\*activator\*\*\*\* (  
\*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\*) is homologous to mammalian serine proteases and  
 contains an apparent activation cleavage site at arginine- \*\*\*\*275\*\*\*\* .  
 To clarify the functional consequences of cleavage at arginine- \*\*\*\*275\*\*\*\*  
 of \*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\* , site-specific mutagenesis was performed to  
 convert arginine- \*\*\*\*275\*\*\*\* to a glutamic acid. The mutant enzyme  
 (designated Arg- \*\*\*\*275\*\*\*\* .fwdarw. Glu \*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\*) could  
 be converted to the two-chain form by Staphylococcus aureus V8 protease  
 but not by plasmin. The one-chain form was 8 times less active against the  
 tripeptide substrate H-D-isoleucyl-L-prolyl-L-arginine-p-nitroanilide  
 (S-2288), and the ability of the enzyme to activate plasminogen in the  
 absence of fibrinogen was reduced 20-50 times compared to the two-chain  
 form. In contrast, one-chain Arg- \*\*\*\*275\*\*\*\* .fwdarw. Glu \*\*\*\*t\*\*\*\* -  
\*\*\*\*PA\*\*\*\* has equal activity to the two-chain form when assayed in the  
 presence of physiological levels of fibrinogen and plasminogen.  
\*\*\*\*Fibrin\*\*\*\* bound significantly more of the one-chain form of \*\*\*\*t\*\*\*\*  
- \*\*\*\*PA\*\*\*\* than the two-chain form for both the wild-type and mutated  
 enzymes. One- and two-chain forms of the wild-type and mutated  
\*\*\*\*plasminogen\*\*\*\* \*\*\*\*activators\*\*\*\* slowly formed complexes with  
 plasma protease inhibitors, although the one-chain forms showed decreased  
 complex formation with .alpha.2-macroglobulin. The one-chain form of  
\*\*\*\*t\*\*\*\* - \*\*\*\*PA\*\*\*\* therefore is fully functional under physiologic  
 conditions and has an increased \*\*\*\*fibrin\*\*\*\* binding compared to the  
 two-chain form.

PY 1987  
 SO BIOCHEMISTRY, (1987) 26 (2), 338-343.  
 CODEN: BICHAW. ISSN: 0006-2960.  
 TI FUNCTIONAL ROLE OF PROTEOLYTIC CLEAVAGE AT ARGinine- \*\*\*\*275\*\*\*\* OF HUMAN  
 TISSUE \*\*\*\*PLASMINogen\*\*\*\* \*\*\*\*ACTIVATOR\*\*\*\* AS ASSESSED BY  
 SITE-DIRECTED MUTAGENESIS.

=> d hist

(FILE 'HOME' ENTERED AT 10:53:16 ON 05 SEP 2002)

FILE 'BIOSIS, MEDLINE, CAPLUS, EMBASE, SCISEARCH' ENTERED AT 10:53:47 ON 05 SEP 2002

L1 166707 S TPA OR T-PA OR (PLASMINOGEN (W) ACTIVATOR)  
 L2 6 S L1 AND (K429? OR H417? OR D477?)  
 L3 3 DUP REM L2 (3 DUPLICATES REMOVED)  
 L4 997 S L1 AND ZYMOGEN  
 L5 364 DUP REM L4 (633 DUPLICATES REMOVED)  
 L6 97 S L5 AND FIBRIN  
 L7 62 S L6 AND PY<=1996  
 L8 2 S L7 AND (275 OR 276)

=> s l5 and salt bridge  
 L9 7 L5 AND SALT BRIDGE

=> s l5 and (salt (w) bridge)  
 L10 7 L5 AND (SALT (W) BRIDGE)

=> s l1 and (salt (w) bridge)  
 L11 60 L1 AND (SALT (W) BRIDGE)

=> dup rem l11  
 PROCESSING COMPLETED FOR L11  
 L12 21 DUP REM L11 (39 DUPLICATES REMOVED)

=> s l12 and py<=1996  
 1 FILES SEARCHED...  
 4 FILES SEARCHED...  
 L13 8 L12 AND PY<=1996

=> d l13 ab au ti py so

L13 ANSWER 1 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 AB Trypsinogen is converted to trypsin by the removal of a peptide from the N terminus, which permits formation of a \*\*\*salt\*\*\* \*\*\*bridge\*\*\* between the new N-terminal Ile (residue 16) and Asp194. Formation of this \*\*\*salt\*\*\* \*\*\*bridge\*\*\* triggers a conformational change in the "activation domain" of trypsin, creating the S1 binding site and oxyanion hole. Thus, the activation of trypsinogen appears to represent an example of protein folding driven by electrostatic interactions. The following trypsin mutants have been constructed to explore this problem: Asp194Asn, Ile16Val, Ile16Ala, and Ile16Gly. The bovine pancreatic trypsin inhibitor (BPTI), benzamidine, and leupeptin affinities and activity and pH-rate profiles of these mutants have been measured. The changes in BPTI and benzamidine affinity measure destabilization of the activation domain. These experiments indicate that hydrophobic interactions of the Ile16 side chain provide 5 kcal/mol of stabilization energy to the activation domain while the \*\*\*salt\*\*\* \*\*\*bridge\*\*\* accounts for 3 kcal/mol. Thus, hydrophobic interactions provide the majority of stabilization energy for the trypsinogen to trypsin conversion. The pH-rate profiles of I16A and I16G are significantly different than the pH-rate profile of trypsin, further confirming that the activation domain has been destabilized. Moreover, these mutations decrease k-cat/K-m and leupeptin affinity in parallel with the decrease in stability of the activation domain. Acylation is selectively decreased, while substrate binding and deacylation are not affected. Together these observations indicate that the stability of protein structure is an important component of transition state stabilization in enzyme catalysis. These results also suggest that active zymogens can be created without providing a counterion for Asp194, and thus have important implications for the elucidation of the structural features which account for the zymogen activity of tissue \*\*\*plasminogen\*\*\* \*\*\*activator\*\*\* and urokinase.  
 AU Hedstrom, Lizbeth (1); Lin, Tiao-Yin; Fast, Walter  
 TI Hydrophobic interactions control zymogen activation in the trypsin family of serine proteases.  
 PY 1996  
 SO Biochemistry, (1996) Vol. 35, No. 14, pp. 4515-4523.  
 ISSN: 0006-2960.

L13 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 AB Tissue-type \*\*\*plasminogen\*\*\* \*\*\*activator\*\*\* ( \*\*\*t\*\*\* -  
 \*\*\*PA\*\*\* ), a multidomain serine proteinase of the trypsin-family  
 catalyses the rate-limiting step in fibrinolysis, the activation of  
 plasminogen to the fibrin-degrading proteinase plasmin. Trigonal crystals  
 have been obtained of the recombinant catalytic domain of human two-chain  
 \*\*\*t\*\*\* - \*\*\*PA\*\*\*, consisting of a 17 residue A chain and the 252  
 residue B chain. Its X-ray crystal structure has been solved applying  
 Patterson and isomorphous replacement methods and has been  
 crystallographically refined to an R-value of 0.184 at 2.3 ANG resolution.  
 The chain fold, active-site geometry and Ile276-Asp477 \*\*\*salt\*\*\*  
 \*\*\*bridge\*\*\* are similar to that observed for trypsin. A few  
 surface-located insertion loops differ significantly however. The  
 disulfide bridge Cys315-Cys384, practically unique to the  
 \*\*\*plasminogen\*\*\* \*\*\*activators\*\*\*, is incorporated without drastic  
 conformational changes as the insertion loop preceding Cys384 makes a  
 bulge on the molecular surface. The unique basic insertion loop  
 Lys296-Arg304 flanking the primed subsites, which has been shown to be of  
 importance for PAI-1 binding and for fibrin specificity, is partially  
 disordered; it can therefore freely adapt to proteins docking to the  
 active site. The S1 pocket of \*\*\*t\*\*\* - \*\*\*PA\*\*\* is almost identical  
 to that of trypsin, whereas the S2 site is considerably reduced in size by  
 the imposing Tyr368 side-chain, in agreement with the measured preference  
 for P1 Arg and P2 Gly residues. The neighbouring S3-S4 hydrophobic groove  
 is mainly hydrophobic in nature. The structure of the proteinase domain of  
 two-chain \*\*\*t\*\*\* - \*\*\*PA\*\*\* suggests that the formation of a  
 \*\*\*salt\*\*\* \*\*\*bridge\*\*\* between Lys429 and Asp477 may contribute to  
 the unusually high catalytic activity of single-chain \*\*\*t\*\*\* -  
 \*\*\*PA\*\*\*, thus stabilizing the catalytically active conformation without  
 unmasking the Ile276 amino terminus. Modeling studies show that the  
 covalently bound kringle 2 domain in full-length \*\*\*t\*\*\* - \*\*\*PA\*\*\*  
 could interact with an extended hydrophobic groove in the catalytic  
 domain; in such a docking geometry its "lysine binding site" and the  
 "fibrin binding patch" of the catalytic domain are in close proximity.  
 AU Lamba, Doriano; Bauer, Margit; Huber, Robert; Fischer, Stephen; Rudolph,  
 TI Rainer; Kohnert, Ulrich; Bode, Wolfram (1)  
 The 2.3 A crystal structure of the catalytic domain of recombinant  
 two-chain human tissue-type \*\*\*plasminogen\*\*\* \*\*\*activator\*\*\* .  
 PY 1996  
 SO Journal of Molecular Biology, (1996) Vol. 258, No. 1, pp. 117-135.  
 ISSN: 0022-2836.

L13 ANSWER 3 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 AB Prothrombin fragment 2 (the second kringle) has been co-crystallized with  
 PPACK (D-Phe-Pro-Arg)-thrombin and the structure of the non-covalent  
 complex has been determined and refined (R = 0.16) at 3.2 ANG resolution  
 using X-ray crystallographic methods. The kringles interact with thrombin  
 at a site that has previously been proposed to be the heparin binding  
 region. The latter is a highly electropositive surface near the C-terminal  
 helix of thrombin abundant in arginine and lysine residues. These form  
 \*\*\*salt\*\*\* \*\*\*bridges\*\*\* with acidic side chains of kringle 2.  
 Somewhat unexpectedly, the negative groups of the kringle correspond to an  
 enlarged anionic center of the lysine binding site of lysine binding  
 kringles such as plasminogen K1 and K4 and \*\*\*TPA\*\*\* K2. The anionic  
 motif is DGDEE in prothrombin kringle 2. The corresponding cationic center  
 of the lysine binding site region has an unfavorable Arg71Phe substitution  
 but Lys35 is conserved. However, the folding of fragment 2 is different  
 from that of prothrombin kringle 1 and other kringles: the second outer  
 loop possesses a distorted two-turn helix and the hairpin beta-turn of the  
 second inner loop pivots at V64 and D70 by 60 degree . The Lys35 is  
 located on a turn of the helix, which causes it to project into solvent  
 space in the fragment 2-thrombin complex, thereby devastating the cationic  
 center of the lysine binding site. Since fragment 2 has not been reported  
 to bind lysine, it most likely has a different inherent folding  
 conformation for the second outer loop, while the movement of the V64-D70  
 beta-turn is most likely a conformational change accompanying  
 complexation, both of which reveal a new heretofore unsuspected  
 flexibility in kringles. The fragment 2-thrombin complex is the first  
 cassette module-catalytic domain interaction determined for a multi-domain  
 blood protein and only the second domain-domain interaction to be  
 described among such proteins, the other being Ca<sup>2+</sup> prothrombin fragment 1  
 (Gla-domain and kringle 1). It has been possible to propose a reasonable  
 model for the four domain prothrombin structure using these two double  
 domain structures.

AU Arni, Raghuvir K.; Padmanabhan, Kaillathe; Padmanabhan, K. P.; Wu,  
Tswei-Ping; Tulinsky, A. (1)  
TI Structure of the non-covalent complex of prothrombin kringle 2 with  
PPACK-thrombin.  
PY 1994  
SO Chemistry and Physics of Lipids, (1994) vol. 67-68, No. 0, pp. 59-66.  
ISSN: 0009-3084.

L13 ANSWER 4 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AB Both human and bovine prothrombin fragment 2 (the second kringle) have been cocrystallized separately with human PPACK (D-Phe-Pro-Arg)-thrombin, and the structures of these noncovalent complexes have been determined and refined (R = 0.155 and 0.157, respectively) at 3.3- Å resolution using X-ray crystallographic methods. The kringles interact with thrombin at a site that has previously been proposed to be the heparin binding region. The latter is a highly electropositive surface near the C-terminal helix of thrombin abundant in arginine and lysine residues. These form \*\*\*salt\*\*\* \*\*\*bridges\*\*\* with acidic side chains of kringle 2. Somewhat unexpectedly, the negative groups of the kringle correspond to an enlarged anionic center of the lysine binding site of lysine binding kringles such as plasminogens K1 and K4 and \*\*\*TPA\*\*\* K2. The anionic motif is DGDEE in prothrombin kringle 2. The corresponding cationic center of the lysine binding site region has an unfavorable Arg70Asp substitution, but Lys 35 is conserved. However, the folding of fragment 2 is different from that of prothrombin kringle 1 and other kringles: the second outer loop possesses a distorted two-turn helix, and the hairpin beta-turn of the second inner loop pivots at Val64 and Asp70 by 60 degree. Lys35 is located on a turn of the helix, which causes it to project into solvent space in the fragment 2-thrombin complex, thereby devastating any vestige of the cationic center of the lysine binding site. Since fragment 2 has not been reported to bind lysine, it most likely has a different inherent folding conformation for the second outer loop, as has also been observed to be the case with \*\*\*TPA\*\*\* K2 and the urokinase kringle. The movement of the Val64-Asp70 beta-turn is most likely a conformational change accompanying complexation, which reveals a new heretofore unsuspected flexibility in kringles. The fragment 2-thrombin complex is only the second cassette module-catalytic domain structure to be determined for a multidomain blood protein and only the third domain-domain interaction to be described among such proteins, the others being factor Xa without a Gla domain and Ca-2+ prothrombin fragment 1 with a Gla domain and a kringle.

AU Arni, Raghuvir K.; Padmanabhan, Kaillathe; Padmanabhan, K. P.; Wu,  
Tswei-Ping; Tulinsky, A. (1)  
TI Structures of the noncovalent complexes of human and bovine prothrombin  
fragment 2 with human PPACK-thrombin.  
PY 1993  
SO Biochemistry, (1993) vol. 32, No. 18, pp. 4727-4737.  
ISSN: 0006-2960.

L13 ANSWER 5 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AB In contrast to most other serine proteases, tissue-type \*\*\*plasminogen\*\*\* \*\*\*activator\*\*\* ( \*\*\*t\*\*\* - \*\*\*PA\*\*\* ) possesses enzymatic activity as the one-chain zymogen form. The hypothesis that lysine residues 277 or 416 may be involved in stabilization of an active conformation of one-chain \*\*\*t\*\*\* - \*\*\*PA\*\*\* via \*\*\*salt\*\*\* - \*\*\*bridge\*\*\* formation with aspartic acid residue 477 was tested by site-directed mutagenesis. Four recombinant \*\*\*t\*\*\* - \*\*\*PA\*\*\* mutants were constructed. The amidolytic activities of these analogues were compared to that of authentic \*\*\*t\*\*\* - \*\*\*PA\*\*\*. Substitution of arginine-275 provided an analogue ([R275G] \*\*\*t\*\*\* - \*\*\*PA\*\*\* ) resistant to plasmin cleavage. The amidolytic activity of [R275G] \*\*\*t\*\*\* - \*\*\*PA\*\*\* was comparable to that of authentic one-chain \*\*\*t\*\*\* - \*\*\*PA\*\*\*, and so was the activity of [R275L,K277L] \*\*\*t\*\*\* - \*\*\*PA\*\*\*, in which additional substitution of lysine residue 277 was carried out. This suggested that its presence was nonessential for obtaining one-chain \*\*\*t\*\*\* - \*\*\*PA\*\*\* activity. In contrast, substitution of lysine residue 416 to obtain [K416S] \*\*\*t\*\*\* - \*\*\*PA\*\*\* and [K416S,H417T] \*\*\*t\*\*\* - \*\*\*PA\*\*\* resulted in substantial quenching of amidolytic one-chain activity. As expected, the amidolytic activities of the two-chain forms were less affected by the substitution. Involvement of lysine residue 416 in one-chain \*\*\*t\*\*\* - \*\*\*PA\*\*\* activity was also indicated by decreased activities of [K416S] \*\*\*t\*\*\* - \*\*\*PA\*\*\* and [K416S,H417T] \*\*\*t\*\*\* - \*\*\*PA\*\*\* with plasminogen as the substrate. The one-chain activity of the lysine residue 416 substitution analogues was partially restored in the presence of fibrin. This could indicate that strong ligands such as fibrin might provide an alternative

stabilization of the active conformation of one-chain \*\*\*PA\*\*\* -

AU\* PETERSEN L C; BOEL E; JOHANNESSEN M; FOSTER D  
TI QUENCHING OF THE AMIDOLYTIC ACTIVITY OF ONE-CHAIN TISSUE-TYPE  
\*\*\*PLASMINOGEN\*\*\* \*\*\*ACTIVATOR\*\*\* BY MUTATION OF LYSINE-416.  
PY 1990  
SO BIOCHEMISTRY, (1990) 29 (14), 3451-3457.  
CODEN: BICHAW. ISSN: 0006-2960.

L13 ANSWER 6 OF 8 MEDLINE

AB The contribution of intersubunit interactions to allosterically induced conformational changes in phosphorylase are considered. Phosphorylase a, Pa (phosphorylated at Ser-14), is significantly in the active (R) conformation, while phosphorylase b, Pb (nonphosphorylated), is predominantly in the inactive (T) conformation. The structure of glucose-inhibited ( \*\*\*T\*\*\* ) \*\*\*Pa\*\*\* has been determined at 2.5-A resolution and atomic coordinates have been measured. These data have been used to calculate the solvent accessible surface area at the subunit interface and map noncovalent interactions between protomers. The subunit contact involves only 6% of the Pa monomer surface, but withdraws an area of 4,600 A<sup>2</sup> from solvent. The contact region is confined to the N-terminal (regulatory) domain of the subunit. Half of the residues involved are among the 70 N-terminal peptides. A total of approximately 100 atoms take part in polar or nonpolar contacts of less than 4.0 A with atoms of the symmetry-related monomer. The contact surface surrounds a central cavity at the core of the interface of sufficient volume to accommodate 150-180 solvent molecules. There are four intersubunit \*\*\*salt\*\*\* \*\*\*bridges\*\*\*. Two of these (Arg 10/Asp 32, Ser-14-P/Arg 43) are interactions between the N-terminus of one protomer with an alpha-helix loop segment near the N-terminus of the symmetry-related molecule. These two are relatively solvent accessible. The remainder (Arg 49/Glu 195, Arg 184/Asp 251) are nearer the interface core and are less accessible. The \*\*\*salt\*\*\* \*\*\*bridges\*\*\* at the N-terminus are surrounded by the polar and nonpolar contacts which may contribute to their stability. Analysis of the difference electron density between the isomorphous Pa and Pb crystal structures reveals that the N-terminal 17 residues of Pb are disordered. Pb thus lacks two intermolecular and one intersubunit (Ser-14-P/Arg 69) salt linkage present in Pa. The absence of these interactions in Pb is manifested in the difference in the free energy of T leads to R activation, which is 4 kcal more than that for Pa. Difference Fourier analysis of the T leads to R transition in substrate-activated crystals of Pa suggests that the 70 N-terminal residues undergo a concerted shift towards the molecular core; \*\*\*salt\*\*\* \*\*\*bridges\*\*\* are probably conserved in the transition. It is proposed that the N-terminus, when "activated" by phosphorylation (via a specific kinase) behaves as an intramolecular "effector" of the R state in phosphorylase and serves as the vehicle of homotropic cooperativity between subunits of the dimer.

AU Sprang S; Fletterick R J  
TI Subunit interactions and the allosteric response in phosphorylase.  
PY 1980  
SO BIOPHYSICAL JOURNAL, \*\*\* (1980 Oct)\*\*\* 32 (1) 175-92.  
Journal code: 0370626. ISSN: 0006-3495.

L13 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2002 ACS

AB Assocn. energies of 3 porphyrins bearing meso-4-pyridinium, -4-anilinium, or -4-benzoate substituents (TPPyP, \*\*\*TPA\*\*\*, TCP) are evaluated in water with 23 different ligands. UV titrns. show excellent fit and const. values (usually +/- .10%) for the equil. consts. K evaluated at different wavelengths as well as isosbestic points, securing 1:1 complexes as well as negligible self-assocn. of the porphyrins. Complexation-induced proton NMR shifts (CIS) show shielding of the ligands by up to -8.0 ppm and of up to -3.4 ppm on the porphyrins with arom. ligands; they agree with tight face-to-face conformations. Ionic binding contributions can be factorized with 5.2 +/- 1.1 kJ per mol and per \*\*\*salt\*\*\* \*\*\*bridge\*\*\* (or ion pair). After subtraction of the \*\*\*salt\*\*\* \*\*\*bridge\*\*\* increments, const. binding free energies are obsd. with .DELTA.Gvdw = 7.2 +/- 1.5 kJ mol<sup>-1</sup> for all benzene derivs. and .DELTA.Gvdw = 15.8 +/- 1.8 kJ mol<sup>-1</sup> for all naphthalene-like, and .DELTA.Gvdw = 18.5 +/- 0.5 kJ mol<sup>-1</sup> for phenanthrene-like derivs. Deviations are obsd. with bulky substituents like +NMe<sub>3</sub> or SO<sub>3</sub><sup>-</sup> groups which allow no close contact between the arom. planes, as evident from CHARMM simulations. Heterocyclic electroneutral ligands show the same const. .DELTA.Gvdw values, independent of no. and position of the heteroatoms. Comparison of 33 different .DELTA.Gvdw contributions with the no. of double bonds occurring in the ligands yields, for the first time, a comprehensive

description of stacking interactions with an increment of  $4 \pm 0.15$  kJ per mol and  $\pi$ -electrostatic measurements with saturated ligands of comparable surface show no  $\Delta G_{\text{vdw}}$  contributions, indicating that the stacking is not driven by solvophobic but by dispersive forces. Copper(II) in the porphyrins (CuTPPyP) leads within  $\pm 0.4$  kJ mol<sup>-1</sup> to the same association energies as without metal, whereas introduction of zinc leads to a decrease by usually 3.4 kJ mol<sup>-1</sup>. Axial orientations of the ligands are observed with complexes of  $\alpha$ , $\omega$ -diaminoalkanes with a gable or sandwich Zn-porphyrin dimer. The  $\Delta G_{\text{cplx}}$  values, measured in chloroform, increase with the match between the Zn and the N atoms. Measurements of K in binary methanol-water mixtures with four complexes (e.g. TPPyP + phenanthroline) show linear correlations with solvent polarity or hydrophobicity parameters. Linear correlations are also found for the first time between the complexation-induced Soret band wavelength changes and the corresponding  $\Delta G_{\text{cplx}}$  values.

AU Schneider, Hans-Joerg; Wang, Manxue  
 TI Supramolecular Chemistry. 49. Ligand-Porphyrin Complexes: Quantitative Evaluation of Stacking and Ionic Contributions  
 PY \*\*\*1994\*\*\*  
 SO Journal of Organic Chemistry ( \*\*\*1994\*\*\* ), 59(24), 7464-72  
 CODEN: JOCEAH; ISSN: 0022-3263

L13 ANSWER 8 OF 8 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.  
 AB Protein C inhibitor (PCI) inhibits multiple plasma serine proteases. To determine which residues contribute to its specificity of inhibition, 19 mutations in the reactive site loop of PCI (from Thr352 to Arg357) were generated and assayed with thrombin, activated protein C (APC), and factor Xa. To identify the intermolecular interactions responsible for these kinetics, a molecular model of PCI was generated using  $\alpha$ 1-protease inhibitor and ovalbumin as templates. This model of PCI was docked with thrombin, followed by extensive energy minimization, to determine a lowest energy complex. The resulting docked complex was used as a template to form molecular models of PCI-APC and PCI-factor Xa complexes. The best inhibitors of thrombin contained Pro or Gly at the P2 position in place of Phe353, with 2- and 7-fold increases in activity, respectively. These substitutions reduced steric interactions with the 60-insertion loop unique to thrombin. The best inhibitors of APC and factor Xa contained Arg at the P3 position in place of Thr352, with 2- and 5-fold increases in inhibition rates, respectively. The molecular model predicts that Arg in this position could form a **salt bridge** with Glu217 of each protease. Changing Arg357 at the P3' position had little effect on protease inhibition, consistent with the observation in the model that this residue points toward the body of PCI, forming a **salt bridge** with Glu220. Given its broad specificity of inhibition, PCI has proven very useful in understanding the nature of serpin- protease interactions using multiple mutations in a serpin assayed with multiple proteases.  
 AU Cooper S.T.; Whinna H.C.; Jackson T.P.; Boyd J.M.; Church F.C.  
 TI Intermolecular interactions between protein C inhibitor and coagulation proteases.  
 PY \*\*\*1995\*\*\*  
 SO Biochemistry, (1995) 34/40 (12991-12997).  
 ISSN: 0006-2960 CODEN: BICHAW

=> d hist

(FILE 'HOME' ENTERED AT 10:53:16 ON 05 SEP 2002)

FILE 'BIOSIS, MEDLINE, CAPLUS, EMBASE, SCISEARCH' ENTERED AT 10:53:47 ON 05 SEP 2002

L1 166707 S TPA OR T-PA OR (PLASMINOGEN (W) ACTIVATOR)  
 L2 6 S L1 AND (K429? OR H417? OR D477?)  
 L3 3 DUP REM L2 (3 DUPLICATES REMOVED)  
 L4 997 S L1 AND ZYMOGEN  
 L5 364 DUP REM L4 (633 DUPLICATES REMOVED)  
 L6 97 S L5 AND FIBRIN  
 L7 62 S L6 AND PY<=1996  
 L8 2 S L7 AND (275 OR 276)  
 L9 7 S L5 AND SALT BRIDGE  
 L10 7 S L5 AND (SALT (W) BRIDGE)  
 L11 60 S L1 AND (SALT (W) BRIDGE)  
 L12 21 DUP REM L11 (39 DUPLICATES REMOVED)  
 L13 8 S L12 AND PY<=1996

=>



---Logging off of STN---

=>  
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	81.11	81.32
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE	TOTAL
	ENTRY	SESSION
CA SUBSCRIBER PRICE	-0.62	-0.62

STN INTERNATIONAL LOGOFF AT 11:10:08 ON 05 SEP 2002